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AMERICAN S CROBIOLOGY

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Abstracts

MAY 30 - JUNE 3, 1999
MCCORMICK PLACE
CHICAGO, ILLINOIS

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OF THE
99TH GENERAL MEETING
OF THE
AMERICAN SOCIETY FOR MICROBIOLOGY

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C-124. Development of an Automated, Amplified Probe Test for the Simultaneous Detection of *N. gonorrhoeae* and *C. trachomatis* on The bioMérieux VIDAS System

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Objective: To develop an automated screening assay with an internal control RNA (IC) for the detection of *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (NG) ribosomal RNA (rRNA). **Methods:** Processed samples are added to disposable VIDAS Probe strips containing amplification and detection reagents. The VIDAS strips are placed in a new instrument (AMPstation) which automates Gen-Probe's Transcription Mediated Amplification (TMA) reaction. CT, NG and IC RNA are simultaneously amplified. The amplified products are detected with enzyme labeled reporter Probes in bioMérieux's VIDAS system. As part of an ongoing study, clinical swab samples (endocervical, vaginal or urethral) were tested and compared to culture or the Gen-Probe PACE CT and PACE NG tests. **Results:** During initial testing with 77 samples, 46 of 47 (97.8%) CT and NG PACE negative clinical swabs were also negative in the VIDAS Probe CT/NG test. One sample was negative with PACE but tested positive twice with the VIDAS Probe CT/NG test. Twenty-nine of 29 PACE positive samples were also positive in the CT/NG test (100%). The remaining PACE negative sample was identified as an amplification failure by the IC system. In a separate experiment with 109 total samples, 66 of 69 (95.6%) CT and NG culture negative clinical swabs were also negative in the VIDAS Probe CT/NG test. Thirty-six of 37 (97.0%) culture positive samples were also positive in the VIDAS Probe CT/NG test. One sample was culture positive but tested negative with the VIDAS Probe *Chlamydia trachomatis* test, PACE and Gen-Probe's AMP CT test. Three samples were culture negative but tested positive with both PACE and VIDAS Probe CT/NG tests. The remaining 3 samples were identified as amplification failures by the IC system. **Conclusion:** The VIDAS Probe CT/NG test is a sensitive method for the detection of CT and NG rRNA. The automated format has the ability to combine immunoassay and probe tests on one system and internal inhibition control in each strips will provide additional tools for the screening of sexually transmitted diseases in clinical labs.

C-125. Use a New Gen-Probe Target Capture Transcription-Mediated Amplification (TMA) Assay for the Detection of *C. Trachomatis* (CT) and *N. Gonorrhoeae* (GC) In Swab and Urine Specimens from Men and Women

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Amplification methods such as Abbott's LCX ligase chain reaction (LCR) offer increased sensitivity in detecting the presence of CT and/or GC as compared to culture methods. We have tested a new generation assay developed by Gen-Probe that uses a dual kinetic chemiluminescent detection system for the simultaneous identification of CT and GC. The Gen-Probe CT/GC assay uses target capture and TMA to purify, concentrate and amplify target rRNA. LCx uses traditional centrifugation and resuspension specimen processing and LCR to prepare and amplify target DNA. Endocervical and male urethral swabs and male and female urine were tested by both methods. Gen-Probe CT/GC assay swab and urine results from 147 female and 93 male patients were compared to LCR results for the same specimen type. Sensitivities for swabs based on these preliminary data were as follows: male CT and GC - 81% (13/16) and 100% (18/18), female CT and GC - 100% (18/18) and 100% (11/11). Urine sensitivities were as follows: male CT and GC - 100% (6/6) and 100% (37/37), female CT and GC - 100% (13/13) and 78% (7/9). Of the female swab results, 9 were Gen-Probe CT or GC swab positive, LCR negative; and 16 female urines were Gen-Probe CT or GC positive, LCR negative. Of the male swab results, 6 were Gen-Probe CT or GC swab positive, LCR negative; and 14 male urines were Gen-Probe CT or GC positive, LCR negative. Preliminary results of efforts to characterize the discordant results are summarized. Testing with target capture-TMA assays for alternate rRNA sites revealed that 7 of the 7 discordant specimens tested to date were positive for CT or GC nucleic acid. Dilution studies of Gen-Probe CT or GC positive male and female urine specimens showed that 6 of 16 remained positive at 1:1000, 9/17 at 1:100, and 14 of 17 at 1:10. None of these samples tested positive at a 1:10 dilution in the LCR-based assay, although 4 specimens showed a slight increase in signal, suggesting near the limit of detection. In addition to these studies, we are investigating the analytical sensitivities of LCR-based specimen processing compared with Gen-Probe target capture on the detection of specimens containing low target levels. In conclusion, these preliminary data show that the Gen-Probe CT/GC assay has excellent sensitivity for LCR positive specimens. Investigations thus far suggest that Gen-Probe CT/GC positive, LCR negative outcomes usually result from falsely negative LCR tests.

C-126. New Gen-Probe Target Capture-TMA Assay Reduces Specimen Inhibition and Increases Sensitivity in STD Testing with Transcription-Mediated Amplification (TMA)

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An novel amplified nucleic acid-based assay has been developed that allows for the specific capture of both *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) rRNA from male and female urine and swab specimens as a sample processing method (Target Capture) prior to) that effectively removes inhibitors of nucleic acid amplification Transcription-Mediated Amplification (TMA). The amplicons are then simultaneously hybridized with two different acridinium ester-labeled oligonucleotides, each specific for its target organism. Differences in the light-off kinetics of the two acridinium ester labels, following a hybridization protection assay, allow deconvolution into signals for each analyte resulting in the detection of the two analytes analytes in one reaction. To test the Target Capture System's efficiency in removing inhibitory substances, naturally occurring and exogenous substances were tested at normal and excessive levels. Gynecological products, salts, minerals and vitamins were not inhibitory to the assay at either level. Blood, a known inhibitor of current amplification assays, did not inhibit performance at up to 40% (v/v) in urine. By concentrating the rRNA targets during processing, Target Capture also offers the potential for extremely high sensitivity. To test this, the 15 CT serovars were assayed at very low levels. Positive results were obtained at levels s... below one elementary body. In a study compar... the GP CT/GC assay to Abbott LCx, both assa... were in 100% agreement for CT positive urines... (n=20), and 100% agreement for the negative... samples (n=179). In a study of male urethral... swabs, the GP CT/GC assay was in 100% agre... ment with culture positive samples (n=52), and... 99.2% agreement for culture negative specimen... (n=118). Target load testing of urine samples ha... shown that a significant number of samples ha... low target loads for GC as well as CT, requiring... an extremely sensitive assay. These data indicate... that the use of next generation nucleic acid... amplification technology improves sample pro... cessing, and yields excellent sensitivity. Couple... with a through-put of at least 200 samples a da... by a single operator, the GP assay has clear... advantages for clinical STD testing.

C-127. Gen-Probe CT/GC Assay on TIGRIS: Full Automation for STD Testing

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TIGRIS is under development as a fully automated nucleic acid amplification system that integrates sample processing, amplification, and detection steps into one instrument. All assay steps, from sample addition to reporting of results, are fully automated and require no operator attendance or intervention. Sample processing is accomplished with Target Capture technology. Specific nucleic acid sequences are captured onto magnetic microparticles. Purified nucleic acids are then amplified isothermally by Transcription-Mediated Amplification (TMA). Amplicon is detected using the Dual Kinetic Assay (DKA), which is capable of detection and differentiation of two analytes in a single reaction. The multiplex Gen-Probe CT/GC Assay is being developed on the TIGRIS system for use with multiple urogenital specimens, including swabs and urines. Initial performance evaluations of the CT/GC assay on TIGRIS were conducted on engineering prototypes and involved testing of a total of 1140 samples. One organism equivalent of CT or GC rRNA (5 fg) was added to each specimen prior to loading on the TIGRIS, which then performed all assay steps. At this low target level, detection of 99% (n = 390) and 90% (n = 750) was demonstrated for CT and GC, respectively. Initial throughput experiments demonstrated time to first result of 3.5 hours for 60 specimens. Protocols with throughput at the planned level of 125/hour are being evaluated. These results demonstrate that full automation of specimen processing, amplification, and detection can be combined with sensitive target detection to yield a system applicable to the clinical STD testing lab.